Genetics of arrhenotokous and thelytokous reproduction in Venturia canescens (Hymenoptera)

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CHAPTER 3

Characterization of fifty-six microsatellite loci in the parasitic wasp *Venturia canescens* with two alternative reproductive modes

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Abstract

Venturia canescens (Hymenoptera: Ichneumonidae) is a solitary endoparasitoid wasp of lepidopteran larvae. It has two alternative modes of haplodiploid reproduction: arrhenotoky and thelytoky. In order to investigate the evolutionary dynamics of coexisting thelytokous and arrhenotokous reproducing individuals an appropriate set of molecular markers is essential. In this study we report the development of 56 microsatellites for both reproductive modes.

Introduction

All Hymenopterans have haplodiploid sex determination. Under arrhenotoky, fertilized eggs are diploid and develop as females; whereas unfertilized eggs remain haploid and develop into males. Under thelytoky males are absent and females develop from unfertilised eggs that undergo diploidy restoration (Crozier, 1977; Beukeboom & Pijnacker, 2000). Although arrhenotoky is the predominant reproductive mode, thelytoky has been observed in all major groups of Hymenoptera. In many insect species thelytoky is induced by endosymbiont microorganisms (Werren *et al.*, 1995; Zchori-Fein *et al.*, 2001; van Wilgenburg *et al.*, 2006). However, in a few cases, a genetic basis has been determined for thelytoky, i.e. for the chalcidoid wasp *Trichogramma cacoeciae* (Stouthamer *et al.*, 1990; Vavre *et al.*, 2004), several species of *Lysiphlebus* (Belshaw *et al.*, 1999), the ant *Plathythyrea puncata* (Schilder *et al.*, 1999), the cape honeybee *Apis mellifera capensis* (Tucker, 1958; Lattorff *et al.*, 2005) and the ichneumonid wasp *Venturia canescens* (Beukeboom & Pijnacker, 2000).

Venturia canescens (Gravenhorst) is an endoparasitoid of pyralid moth larvae (Beling, 1932; Salt, 1976) that has been widely used as a biological model in behavioural, population dynamics, genetic and physiological studies (references in Beukeboom & Pijnacker, 2000; Thiel *et al.* 2006). Beukeboom and Pijnacker (2000) and Mateo Leach *et al.* (Chapter II) showed that thelytoky in *V. canescens* is not due to infection with *Wolbachia* or any other prokaryotic endosymbiont. These studies indicate that parthenogenesis in *V. canescens* indeed has a genetic basis. In Southern Europe both arrhenotokous and thelytokous populations have been found to occur in sympatry (Schneider *et al.*, 2002). These authors studied the genetic variation of both reproductive modes using AFLP and RFLP markers (Schneider *et al.*, 2002; Schneider, 2003). They found that thelytokous wasps form one widespread clone and only in few cases thelytokous females appear closely related to the sympatric arrhenotokous ones. They confirm that this pattern is due to occasional gene flow between the two reproductive modes and not to recurrent arisal of thelytoky from sympatric arrhenotokous populations. However, better indicators of genetic exchange between both reproductive modes and more suitable markers for individual characterisation in population genetic studies are required. Currently, there is a limited set of microsatellites that have been developed for thelytokous *V. canescens* (S. Hubbard and R. Butcher, University of Dundee, personal communication), but many of them do not consistently amplify in arrhenotokous individuals. Here we describe the characterization of a set of microsatellites for *V. canescens* that can reliably be amplified in both reproductive modes.

Materials and Methods

Genomic DNA from 10 arrhenotokous female wasps collected at Mont Boron in 1999 (Schneider *et al.*, 2002) was extracted using a standard proteinase K/salt-chloroform protocol. Presence and quality of DNA was checked on a 1% agarose gel. A microsatellite genomic library was constructed according to the SSR capture enrichment method of Connel *et al.* (1998) by Baseclear B.V. (Leiden, the Netherlands). DNA was fractionated by nebulization and ligated to adapters (oligo AP-11: 5′-CTCTTGCTTAGATCTGGACTA-3′ and oligo AP-12: 5′-TAGTCCAGATCTAAGCAAGAGCACA-3′). Selection for adapter ligated fragments was done by PCR amplification with AP-11 adaptor as primer. Biotinylated repeat oligos (GAG, CTA, CAT, CAC, GAG and CAAC) were used to hybridize and isolate repeat sequences from the fragmented DNA. The fragments containing repeat sequences were isolated using streptavidin-coated paramagnetic beads (Promega). One microliter of the selected fragments was amplified using AP-11 oligo, ligated into pCRII vectors (Invitrogen) and transformed into *E.coli* DH10B cells according to Sambrook *et al.* (1989). Positive clones were selected for PCR amplification using M13 forward or reverse primers and the resulting fragments were sequenced on an Applied Biosystems DNA analyzer 3730 using Big Dye terminator V3.1 (Applied Biosystems). To characterize the repeats in the clone sequences, these were further analyzed using the Tandem Repeat Finder software (Benson, 1999). On the selected sequences, primers were designed with software PRIMER 3 (Rozen & Skaletsky, 2000).

Fourteen arrhenotokous individuals (10 males and 4 females representing five populations from Southern France) and 24 thelytokous females (representing four populations from Southern France and Spain) of *V. canescens* were genotyped for those loci that produced a fragment of the expected size. The forward primers were labeled with a fluorescent dye (HEX, FAM or NED). PCR reactions were performed in 1X PCR buffer magnesium free (Promega) with 2.5 mM $MgCl_2$, 0.2mM dNTPs (Roche), 0.2µM of each primer, 0.4 units of Taq polymerase (Promega) and approximately 5ng of template DNA. The PCR profile was 1 cycle of 3 min at 94°C followed by 35 cycles of 1 min at 94°C, 30 sec at the primer specific temperature (see Table 3.3), 1 min at 72°C and a final cycle of 7 min at 72°C. Reactions were carried out in a GeneAmp 9700 PCR machine from Applied Biosystems. PCR products were resolved on an 8% denaturing acrylamide electrophoresis gel on an ABI prism 377 automatic sequencer. Allele sizes were determined using Rox-500 size standard. The size of the fragments was calculated using GeneScan 3.1 software (Applied Biosystems). Differences in allele number between the two reproductive modes were assessed by Wilcoxon's matched pairs test using package Statistica (StatSoft, OK, USA).

Results

Without performing the high density colony filter step described in Connel *et al.* (1998) a 50-70% enriched library was obtained. We analyzed 287 sequenced fragments to identify the repeat motif of putative microsatellites. Fifty-nine sequences (20.6%) contained no repeat motif and 85 (29.6%) were excluded due to unsuitable repeat size i.e. mononucleotide repeats or repeats larger than four nucleotides (Table 3.1). Hundred and forty-three sequences (49.8%) contained a repeat motif of di-, tri- or tetranucleotide. About 50% of these sequences had one microsatellite motif, but the others had two (25%), three (16.7%) or four and more (8.3 %) repeat types. Fifty-three percent of the microsatellites were dinucleotides, 32.5% trinucleotides and 14.6% tetranucleotides. The proportion of perfect repeats (according to Weber's definition, Weber, 1990) was 46.6%. The most common dinucleotide motifs were TC/AG (39%) and CT/GA (24.8%) (Table 3.2). Many different trinucleotide repeats were represented in the library: CAA/GTT (9.3%) and AGA/TCT (7%) being the most abundant ones. We also found a large diversity of tetranucleotide repeats of which GTGC/CACG and ATCT/TAGA were the most abundant ones (12.5 %). Our results are in contrast with the most predominant motif found by Butcher *et al.* (2000) which was GT, but consistent with motifs in other

Table 3.1. Overview of microsatellite cloning and sequencing in *V.canescens*.

	Number	Ratio (%)
Clones lacking repeat	59	20.6
Excluded clones (no relevant repeat)	85	29.6
Clones with repeat	143	49.8
Total number of clones	287	
Clones with 1 repeat	71	49.7
Clones with 2 repeats	36	25.2
Clones with 3 repeats	24	16.8
Clones with 4 repeats or more	12	8.4
Total number of clones with repeat	143	
Dinucleotide repeats	142	53.0
Trinucleotide repeats	87	32.5
Tetranucleotide repeats	39	14.6
Total number of repeats	268	
Perfect repeats	125	46.6
Imperfect repeats	143	53.4
Total number of repeats	268	

Hymenoptera (Estoup *et al.,* 1993). Hundred microsatellite sequences were chosen for further analysis according to their repeat: the minimum number of repeats was 8 for the di- and 3 for tri- and tetranucleotide motifs.

Table 3.2. Microsatellite motifs in *V.canescens*.

Motif	number	%	Motif	number	%
Dinucleotide			**Tetranucleotide**		
TC/AG	55	38.7	GTGC/CACG	3	7.7
CT/GA	40	28.2	ATCT/TAGA	3	7.7
GT/CA	25	17.6	TGTT/ACAA	3	7.7
TG/AC	16	11.3	TCCC/AGGG	2	5.1
CG/GC	3	2.1	AAAG/TTTC	2	5.1
TA/AT	3	2.1	AAGC/TTCG	2	5.1
Trinucleotide			TATC/ATAG	2	5.1
CAA/GTT	9	10.3	GAAA/CTTT	2	5.1
AAT/TTA	7	8.0	AAGA/TTCT	2	5.1
AGA/TCT	7	8.0	AGCG/TCGC	1	2.6
ACG/TGC	5	5.7	ACAC/TGTG	1	2.6
AGG/TCC	5	5.7	GGAG/CCTC	1	2.6
CTA/GAT	4	4.6	AAGT/TTCA	1	2.6
CAG/GTC	4	4.6	AAGG/TTCC	1	2.6
ACT/TGA	4	4.6	GAGG/CTCC	1	2.6
TCG/AGC	4	4.6	ATTT/TAAA	1	2.6
GGC/CCG	3	3.4	CTTC/GAAG	1	2.6
GAG/CTC	3	3.4	AAAC/TTTG	1	2.6
AGT/TCA	3	3.4	TGCC/ACGG	1	2.6
TTG/AAC	3	3.4	GACG/CTGC	1	2.6
TAT/ATA	3	3.4	TGAG/ACTC	1	2.6
CGT/GCA	3	3.4	AATA/TTAT	1	2.6
TGT/ACA	3	3.4	ACCC/TGGG	1	2.6
GAC/CTG	3	3.4	AATC/TTAG	1	2.6
CAT/GTA	2	2.3	TAAC/ATTG	1	2.6
TTC/AAG	2	2.3	ACGA/TGCT	1	2.6
TAG/ATC	2	2.3	GCTC/CGAG	1	2.6
TAA/ATT	2	2.3			
CCT/GGA	1	1.1			
GTG/CAC	1	1.1			
ACC/TGG	1	1.1			
GAA/CTT	1	1.1			
GCG/CGC	1	1.1			
GCT/CGA	1	1.1			

Six clones lacked enough flanking region to design primers and 38 failed to optimize for PCR or gave dubious amplification patterns, and were discarded, leaving a total of 56 putative microsatellite loci. Thirty-three of these loci were polymorphic on the arrhenotokous genotyped individuals. The number of alleles ranged from 1 to 7 with an average of 2.6 alleles per locus and an overall number of 147 alleles. Thirty of the 56 loci were checked in 24 thelytokous individuals. The number of alleles per locus ranged from 1 to 5 with an average of 2.26 alleles per locus and an overall number of 68 alleles. The arrhenotokous sample had a significant higher number of alleles than the thelytokous one (Wilcoxon's matched pairs test, $Z = 3.32$, $p = 0.001$).

Discussion

Despite more individuals were tested in the thelytokous sample, the number of alleles per locus is lower than in the arrhenotokous sample and thelytokous females are more homozygous than arrhenotokous ones. This is consistent with the mechanism of diploidy restoration that occurs in the thelytokous strains of *V. canescens* (central fusion automictic parthenogenesis, Beukeboom & Pijnacker, 2000). This mechanism enables heterozygosity to be maintained for loci close to the centromere, but loci distal from chiasmata will become homozygous over generations depending on the segregation pattern. The development of microsatellites for *V. canescens* will be a useful tool for the study of the genetic structure of arrhenotokous and thelytokous populations as well as for the consequences of parthenogenesis on the genetic variation in this species.

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Table 3.3. Characterization of 56 microsatellites loci for the parasitic wasp *Venturia canescens*. Size: expected size of the sequenced allele (bp); Repeat motif: repeat comprised between the primers; Tm: annealing temperature (ºC); Primer sequences: forward and reverse 5′-3′ primer sequence. Arrhenotokous (N = 14, 4 females and 10 males) and thelytokous (N = 24 females) alleles and the number of alleles per locus (N_A).

Locus	Accession No.	Size (bp)	Repeat Motif	Tm (ºC)	Primer sequence (5′ – 3′)	Arrhenotokous Alleles	N_A	Thelytokous Alleles	N_A
Vcan060	GU053668	244	(GA)9	58	F: GGAGTTACTGAAGGCAAACAAGG R: AATGACTGAACGGGACTCGAT	253 247	2	247 249	2
Vcan061	GU053669	186	(CT)13	55	F: TACGGTACTGAGAGATGTGTGGA R: TCGGTTAGAAGTGAGACCTGAAC	188 190 200	3	-	-
Vcan062	GU053670	254	(CT)8	55	F: TGACTCCCTATGCACTTCTTCTC R: GAAGGTGTCGAGATATGGTGAAC	250 260 266	3	250 256	2
Vcan063	GU053671	179	(AG)39	55	F: CGTATACTCACGCACACACAAAG R: CTTGACGATATGGGTTGATGG	178 182 184 186	4	168 182	2
Vcan064	GU053672	288	Complex*	55	F: GTTGCTAACTTCGAGGACAGACT R: AGTAGTAACGTTCGATGGCAGAG	280 286	2	-	-
Vcan065	GU053673	218	(TGC)15	55	F: TCATTGTCACTGGTCGGTGT R: GATCATAGGCAACAGCAGCA	209 212 215 218 227 233	6	215 227	2
Vcan066	GU053674	245	(CTTT)11	55	F: GATACCAGACTCGAGATCTATTCAA R: CGACCGACAATCAAGGTTTT	238 240 242 250 252	5	244	1
Vcan067	GU053675	155	(CAA)26	55	F: ATGGTTCAGCAGCAACATCA R: GTTCCCTTGTAAAGCGGATG	138 141 151 154 157	5	148 151 154 157	4
Vcan068	GU053676	155	(TTCG)21	58	F: TCCCGACTTTCTCACTCCTC R: AGGAAGGAAGGAACGAAGGA	141 161 165 169 177	5	-	-
Vcan069	GU053677	228	(AAC)45	55	F: GAATGAGGATCAGCAAAATCG R: GATGGCAGAGCAACTCGTTT	200 218 220 226	4	-	-
Vcan070	GU053678	228	(GCT)10	55	F: TGCTCGCCCTTTTCTTTATT R: CATCTGCCACGACTCTCAAG	221 224	2	227 230	2
Vcan071	GU053679	232	(CAA)11	55	F: CTCCTACGCACTCCCTTCAC R: TTGTACGTTGGCACTTGAGC	231 234 237 240 252	5	222 225 231	3
Vcan072	GU053680	196	(GAC)14	55	F: TGAATTTGTCGTTGCTGCTC R: CGAGGAAGTTCAGGCTCAAG	200 203	2	-	-

* (CAA) CAG (CAA)8 CAG (CAA) CAG (CAA)4 CAG CAT (CAA)4 CCA (CAA)2 (CAG)4 (CAA) CAG (CAA)2 CAC (CAG)2 CAA CAG (CAA)3 CCA

Table 3.3. Continued.

Locus	Accession No.	Size (bp)	Repeat Motif	Tm (°C)	Primer sequence (5′ – 3′)	Arrhenotokous Alleles	N_A	Thelytokous Alleles	N_A
Vcan073	GU053681	244	(TGT)15	60	F: GGTCCAACGGTACTTCCTGA R: ACTTCCGTCAGCCCTACCTT	206 224 245 248 254 260	6	242 245 248	3
Vcan074	GU053682	198	(AGA)8	61	F: CCGAAGCTGAAGAAATCGAA R: CTACAGAGAGGGGCAAATCG	200	1	-	-
Vcan075	GU053683	193	(CAA)10	60	F: TCGTCGGATCAACACAATTT R: TCAAGAATTCGGGAACATCC	193	1	190 193	2
Vcan076	GU053684	154	(TGA)16	60	F: TTGACGCCGTCGAACTAATA R: GTGAAACTGGAGCCTTTGGA	156	1	-	-
Vcan077	GU053685	195	(GAC)3	62	F: GGTGGATTGACGTGCCTTAT R: CCCTCCCCACTTCTCTCTTT	196	1	193 196	2
Vcan078	GU053686	170	(ATT)5	61	F: AGGTGATGTTAGGCGGTTTG R: TTTTCGCGGGTTTTGTTTAC	168 171	2	168	1
Vcan079	GU053687	157	(GA)15	61	F: AGGAACGCAAAATGAAATGG R: TCGTTCGAACTTTTCCCCTA	146 156 158	3	154 160	2
Vcan080	GU053688	181	(AGG)6	63	F: TCCATCTTCGCTCTTTTTCC R: CTTTCTCTTCGCGCTCACTT	181	1	181	1
Vcan081	GU053689	249	(TGC)6	60	F: GCTAGACTCCACGGCTACCA R: CAATAAGGGGCAATTCGTGA	251	1	-	-
Vcan082	GU053690	169	(CAA)8	63	F: GCAACTCCTGCAACAACAGA R: GTGATGAGGGTAGCCGATGT	169	1	169 172	2
Vcan083	GU053691	250	(TGA)11	60	F: ATAGCTCATCGCTCCTCTGC R: CGCCCATCTTGTGCTTATGT	241 244 247	3	241 247 250	3
Vcan084	GU053692	250	(AGA)7	60	F: ACTCGATTTGCGTGGAAAAA R: TTTCGCTTGTTGCTGAGTTC	250	1	-	-
Vcan085	GU053693	199	(TTC)8	53	F: AGGTTCAATGGCTTTGCTGT R: GCTTTCCGAGCTTTTCCTCT	146 161 197 200	4	197 200	2
Vcan086	GU053694	244	(AC)24	60	F: AGCAAACGGGACTTGAATTG R: GGGACTCCAAACCCTCTTGT	245	1	-	-
Vcan087	GU053695	165	(GT)23	55	F: CTTCTAGCTCCGTCGGTGTC R: CGGTGGTTGTTGTCGAGTTA	165	1	165	1

Table 3.3. Continued.

Locus	Accession No.	Size (bp)	Repeat Motif	Tm (°C)	Primer sequence (5′ – 3′)	Arrhenotokous Alleles	N_A	Thelytokous Alleles	N_A
Vcan088	GU053696	198	(CA)44	60	F: AGTAACCGGTCAGCCTTTGG R: CACGTTCCAATTTCCACACA	132 136 142 144	4	132 134 136 142	4
Vcan089	GU053697	234	(TC)33	50	F: GGCCATGTTTCCTACTTCCA R: GGCGGAAAGATTTTTCGATAG	190	1	-	-
Vcan090	GU053698	243	(CT)37	55	F: ATGCGGATGCGTAAGGATAG R: GCTGCTTAACGTTTCGGTCT	251	1	-	-
Vcan091	GU053699	184	(GA)32	60	F: GTAGGCACGTACCGAGGAAA R: TCCACGCTCGTGTGTGTACT	158 170 174 186	4	154 158	2
Vcan092	GU053700	210	(TTCC)6	60	F: CGTTCGTTCTTTCGTTCGTT R: CGGCATTGTCCTTCTTGTTT	208 212 216 220	4	212 216 220	3
Vcan093	GU053701	201	(TC)21	50	F: CTACCAGCACGAGAAGCTGA R: TTTCTCGGTGCTTCTCCACT	201	1	-	-
Vcan094	GU053702	152	(CT)26	60	F: ACGATCGCTCAATCGAAGTT R: CTCCCATAAACTCGGAGCAA	156 158	2	154 156	2
Vcan095	GU053703	100	(CA)24	50	F: GTAATCATTTTCGCTCCGTGA R: TCGTTTCTCTTTTCGTTCGAG	080 092 096	3	-	-
Vcan096	GU053704	175	(AG)39	55	F: CTCACGCACACACAAAGTCC R: TGCTTGACGATATGGGTTGA	172 176 178	3	176	1
Vcan097	GU053705	140	(GA)15	55	F: AATGGAGACAACGAGGCAAC R: ATCAGAGTCGACCCAGCAAC	140 142	2	140 152	2
Vcan098	GU053706	233	(GT)15	50	F: CAATTCGGAAACACTGCAAA R: CCGCTCGATCTTTTCATTTC	233	1	-	-
Vcan099	GU053707	123	(AG)12	55	F: TGGCCATAACAGGGAGAAAG R: GTCACTGGGGAAGAGTGGTG	126 128 130 138	4	126	1
Vcan100	GU053708	224	(GA)12	60	F: ACGGTCAAGTTACCCAAGCA R: GACCAAGCTCCGGATAAACA	215	1	-	-
Vcan101	GU053709	200	(CT)15	45	F: ATCTGGACTAAGCGCGAGAG R: CAACGAGAGGGAAAGAGACG	190	1	-	-
Vcan102	GU053710	174	(TC)14	60	F: TTCCAATTCACGAATCAACG R: CCTCTGAGTCACCGAAAAGC	161 165 169 175	4	161 169 172 175 177	

Table 3.3. Continued.

Locus	Accession No.	Size (bp)	Repeat Motif	Tm (°C)	Primer sequence (5′ – 3′)	Arrhenotokous Alleles	N_A	Thelytokous Alleles	N_A
Vcan103	GU053711	206	(TC)10	48	F: CTCAAGCTATGCATCCAACG R: TCTCGGAGTCAATCCCACTC	154	1	-	
Vcan104	GU053712	164	(GA)14	55	F: CAAAAGGGAGGGAAAGGAAG R: CCCACGTTTTCGGTGTACTT	164	1	-	
Vcan105	GU053713	188	(TC)15	48	F: TGGGCAATTACCCCACTAAA R: GCACGTGCAATTCTGATGAC	156	1	-	
Vcan106	GU053714	197	(TC)24	60	F: CCTCATCTCGAGGGAGGATT R: ATCGCGAGTTGCGTAGTTTC	190 196 206 208 210 214 224	7	186 188 190 194 210	
Vcan107	GU053715	282	(AC)13	60	F: GTCGCCGGCTCATATTTTTA R: AGCAAGTCTGCGATCTTTCC	283	1	-	
Vcan108	GU053716	188	(TC)24	50	F: GGATACACGAACCTGGCATT R: AGACCGAGAGAGGAGGAAGG	190	1	-	
Vcan109	GU053717	192	(AG)10	60	F: TTAATTGAACGGGGAAAACG R: GCAGTCGGTGTAGCGTGTTA	188 190 194	3	-	
Vcan110	GU053718	172	(AC)25	55	F: CCATTCATTCGGATCTCACC R: CCGACGTTTGTATCTTCGTTC	173 175	2	-	
Vcan111	GU053719	264	(ACC)12	55	F: CCACACGAACAATGTCAAT R: CCGTTTTTATGAGCGTAGAG	264	1	-	
Vcan112	GU053720	159	(TC)15	55	F: GCAGAGATTTTTGCCACAGG R: TGGCTGGATGAAGGGATATT	143 149 151 172 176 178	6	143 149 161	
Vcan113	GU053721	199	(GA)11	55	F: TTCAGGGAGGATAGAACGTA R: TCTCTCTCCTTCCCTTTCTC	199	1	199	
Vcan114	GU053722	241	(AG)10	55	F: AAAAATGAACGACAGAAGGA R: GTTGCGCTCTTTGTGAATA	237 241 245 247 251	5	-	
Vcan115	GU053723	106	(CT)11	55	F: TTTTTCACTCTTCGTTCCTC R: TGCTACCCTCTTGATCTCC	096 104 106 124	4	104 110